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Jacobs Journal of Genetics

Research Article

# Association between C677T MTHFR Polymorphism and H pylori Infection among Jordanian Gastric Cancer Patients

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*Received: 05-15-2015*

*Accepted: 06-04-2015*

*Published: 06-09-2015*



## Abstract

**Introduction:** Methyl tetrahydrofolate reductase (MTHFR) polymorphism and *H pylori* infection increase gastric cancer (GC) development. So the aim of this study to evaluate C677T MTHFR gene polymorphism and investigate the possible interaction between this polymorphism and *H pylori* infection as a possible risk factors for GC.

**Material and methods**: This study enrolled one hundred and twenty GC patients from Ibn-Nafees hospital Irbid (2010-2015) and one hundred and thirty eight age matched control with no family history of any cancer. DNA was extracted using DNA extraction and purification kit (Promega, USA). MTHFR C677T was detected by FV-PTH-MTHFR Strip Assay (ViennaLab, Austria). *H pylori* IgG was detected by ELISA (Novalisa, Germany).

**Results**: This case control study showed that MTHFR C677T genotypes frequencies among Jordanians GC is: CC, 61.7%, CT, 34.2%; and TT, 4.2% among GC cases and 54.4%, 37.7% and 7.9% among controls; respectively. The data of this study also showed that the frequency of intestinal GC (42/120=35%) were more common than diffuse GC (26/120=21.7%) among Jordanian GC patients. MTHFR C677T polymorphism increased the risk of GC by 35% (OR =1.35, 95% CI = 0.82 – 2.22), and increase intestinal GC risk by 51% and diffused GC by 34%. MTHFR 677CT and MTHFR 677 TT genotypes interacted with *H pylori* infection and significantly associated with increased risk of GC (*p=0.009* and *p=0.009*; respectively)

**Conclusion:** MTHFR C677T polymorphism increased the risk of GC by 35%. Both MTHFR 677CT and MTHFR 677 TT genotypes interacted with *H pylori* infection are significantly increased the risk of GC among Jordanian.

**Key words**: C677T; MTHFR; *H pylori*; Gastric Cancer

## Introduction

Methyl tetrahydrofolate reductase (MTHFR) is a key enzyme for folate metabolism. MTHFR gene encoded 5-10-methylentetrahydrofolate reductase enzyme that catalyze the reduction of 5-10 methylentetrahydrofolate to 5-methylentetrahydrofolate which is a co-substrate for the re-methylation of homocysteine into methionine [1], and finally to S-adenosyl-L-methionine. Reduced MTHFR gene activity result in low S-adenosyl-L-methionine activity that increased cancer risk [2]. Understanding MTHFR gene polymorphism may enhance understanding the pathogenesis of cancer. MTHFR polymorphism are associated with colorectal cancer [3], esophageal squamous cell carcinoma [4], chronic myeloid leukemia [5], breast cancer [6] and thyroid carcinoma [7]. Two functional polymorphisms were recorded within MTHFR gene; C677T and A1298C [8]. C677T result in a substitution of alanine for valine at amino acid 222 in exon 4 and low enzyme activity.

The epidemiology of these polymorphism varies dependent on the geography and ethnicity [9].

*H pylori* is a microaerophilic, Gram negative, spiral shaped rod that colonizes the stomach. It is recognized as a carcinogen and the most common etiologic agent of infection related cancer [10]. *H pylori* usually causes asymptomatic gastric infection that may be transformed with time into chronic gastritis which destruct stomach mucosa (atrophic gastritis) and usually end up with permanently acid-free stomach [11]. Multistep gastric carcinogenesis may result from the reactive oxygen species that are released from activated neutrophils induced by *H pylori* that bind the host nucleic acids, and turning them into mutated forms. *H pylori* infection may also induces epigenetic transformations, and aberrant expression of microRNAs are also linked to gastric tumor genesis [12]. Most *H pylori* infections acquired via oral-oral or oral-fecal route and infection outcome depend on the host-environment interaction that depend on bacterial strain, host genetic diversity [11] and presence of antibodies for the CagA protein that are markers for an increased risk of gastric adenocarcinoma [13].

Stomach cancer incidence is second only to lung cancer worldwide, with an estimated 870,000 new cases and 655,000 deaths every year [14]. There is major geographical differences among different countries. High-risk areas are East Asia, South America and Eastern Europe. GC in Jordan constitutes 3.2% of new cancer cases [15]. Multiple factors play a role in gastric cancer development including *H pylori* infection, diet life style, and genetic predisposition. The prevalence of *H pylori* ranges from 25% in developed countries to 80-90% in developing countries [15]. Association between chronic *H pylori* infection and GC is well established; 79% of Jordanian GC patients were *H pylori* infected [15]. The association of C677T MTHFR with susceptibility to gastric cancer were inconsistent; some studies reported that MTHFR C677T modulate and increased an individual's susceptibility to gastric cancer [16], especially when combined with cigarette smoking and with a low intake of fruit and vegetables [17], whereas others reported that MTHFR C677T polymorphisms by themselves do not play any role in the etiology of stomach cancer among Korean population [18]. So, this case-control study is conducted among Jordanian GC patients to evaluate C677T MTHFR gene polymorphism and investigate the possible interaction between this polymorphism and *H pylori* infection as a possible risk factor for GC.

## Material and Methods

This case control study enrolled one hundred and twenty GC patients from Ibn-Nafees hospital Irbid (2010-2015) and one hundred and thirty eight age matched control with no family history of any cancer. All GC patients were clinically and histopathologically diagnosed and TNM staged. Gastric cancer was classified by anatomical site (cardia or non-cardia) and histological types (intestinal, diffuse). Control enrolled one hundred and thirty eight healthy individuals referred to the same hospital who were cancer/ ulcer free and during the same period. Consent form was signed by all participants before interviewing and sample collection. DNA was extracted from blood samples using DNA extraction and purification kit (Promega, USA). MTHFR C677T was detected from the extracted DNA using reverse-hybridization by FV-PTH-MTHFR Strip Assay (ViennaLab, Austria), which depend on three successive steps including multiplex DNA isolation, *in vitro* DNA amplification and hybridization to the test strip.

*H pylori* IgG from blood samples were detected by ELISA in microplates coated with *H pylori* antigen (Novalisa, Germany) at 450nm and 620nm wavelength absorbance capability (BioRad, USA) and according to the manufacturer instructions.

Statistical analysis carried out using the Statistical Package for Social Sciences (SPSS) version 20. The association between MTHFR C677T and gastric cancer risk was evaluated by calculating the odd ratios (OR) using "Mantel Haenszel"method and 95% confidence intervals (CI).

**Table 1**. MTHFR C677T variants stratified by subtype and subsite.

| Subsite no (%) | | Subtype no (%) | | GC no (%) 120 | Control no (%) 138 | MTHFR variant |
|---|---|---|---|---|---|---|
| Non-Cardia no=52 | Cardia no=39 | Diffuse no=26 | Intestinal no=42 | | | |
| 29(55.8) | 21(53.8) | 16(61.5) | 27(64.3) | 74(61.7) | 75(54.4) | CC |
| 21(40.4) | 15(38.5) | 8(30.7) | 11(26.1) | 41(34.2) | 52(37.7) | CT |
| 2(3.8) | 3(7.7) | 2(7.7) | 4 (9.5) | 5(4.2) | 11 (7.9) | TT |
| 23(44.2) | 18(46.2) | 10(38.5) | 15(35.7) | 46(38.3) | 63(45.7) | T carriers |

**Table 2**. Association MTHFR C677T polymorphism with gastric cancer stratified by anatomic subsite and histological subtype.

| | MTHFR variant | Case/control | OR(95% CI) |
|---|---|---|---|
| GC patients | CC | 74/75 | Ref |
| | T carriers | 46/63 | 1.35(0.82 - 2.22) |
| Intestinal GC | CC | 27/75 | Ref |
| | T carriers | 15/63 | 1.51(0.74 - 3.08) |
| Diffuse GC | CC | 16/75 | Ref |
| | T carriers | 10/63 | 1.34(0.57 - 3.17) |
| Cardia GC | CC | 21/75 | Ref |
| | T carriers | 18/63 | 0.98(0.48 – 2.0) |
| Non-cardia GC | CC | 29/75 | Ref |
| | T carriers | 23/63 | 1.06(0.56 - 2.01) |

**Table 3**. Association between MTHFR C677T variants and *H pylori* infection.

| | MTHFR variant no (%) | | $\chi 2$ | *p-value* |
|---|---|---|---|---|
| | CC GC n=74 | CC control n=75 | | |
| *H pylori* positive | 51(68.9%) | 43(57.3%) | 3.09 | 0.79 |
| *H pylori* negative | 23(31.1%) | 32(42.7%) | | |
| | CT GC n=41 | CT control n=52 | | |
| *H pylori* positive | 31(75.6%) | 27(51.9%) | 11.02 | 0.009 |
| *H pylori* negative | 10(26.2%) | 25(48.1%) | | |
| | TT GC n=5 | TT control n=11 | | |
| *H pylori* positive | 4(80%) | 6(54.5%) | 14.86 | 0.001 |
| *H pylori* negative | 1(20%) | 5(45.5%) | | |

## Results

The frequencies of MTHFR C677T genotypes among GC and control, and the frequencies of GC anatomical subsite and histostological subtypes are also shown in table (1).

GC of MTHFR C677T showed 35% increased risk of gastric cancer compared to the wild type (table 2), (OR = 1.35, 95% CI=0.82 - 2.22), 51% increased risk associated with intestinal GC (OR =1.51, 95% CI = 0.74 - 3.08) and 35% increased risk among diffused GC (OR= 1.34, CI = 0.57 - 3.17).

The results of this study showed (table 3) that *H pylori* infection are significantly associated with gastric cancer among MTHFR CT genotype (*p=0.009*) and TT genotype (*p=0.001*).

## Discussion

GC is the third leading cause of cancer deaths worldwide, and 80% of the 1 million new cases annually are caused by a treatable *H pylori* infection [14]. MTHFR C677T polymorphism plays an important role in GC pathogenesis. Among Asians and Caucasians, MTHFR C677T polymorphism is a strong risk factor for GC, this evidence retrieved from twenty-five case-control studies with 6,572 cases and 9,584 controls [19]. The data about Jordanian GC prevalence, genetics, histology and anatomy are lacking, but Abbasi et al [15] showed younger presentation age (median age 61.2 years) among GC Jordanian patients compared to United States patients with 82% prevalence in his report compared to 50% prevalence at age of 50 among united states GC patients. What makes this study unique is that, up to our knowledge this is the first study that examine MTHFR C677T gene polymorphism as a risk factor for GC among Jordanians.

The results of this study shows that MTHFR C677T genotypes frequencies among Jordanians GC patients are: CC, 61.7%, CT, 34.2%; TT, 4.2% and are 54.4%, 37.7% and 7.9% among controls; respectively. These frequencies are similar to MTHFR C677T genotypes reported by Al-Motassem et al among lung cancer patients [8], where MTHFR C677T frequencies among lung cancer cases; CC, 59.6%, CT, 33% and TT, 7.4% and 49.4%, 40.2% and 10.3% among controls; respectively. According to the histological type of GC, the data of this study showed that the frequency of intestinal (well differentiated) GC (42/120=35%) are more common than diffuse (non-differentiated) GC (26/120=21.7%). Intestinal GC diagnosed in high-risk populations and is more likely to be sporadic than inherited [20] and strongly associated with *H pylori* infection. The mechanism whereby chronic *H pylori* infection may leads to gastric cancer is unclear, one of the mechanisms is that chronic bacterial infection may lead to non-atrophic gastritis that transforms into atrophic gastritis then into intestinal metaplasia and dysplasia [21]. The other suggested mechanism is elevation of cycloxgenase-2 COX-2 in gastric carcinomas [22]. According to the location, the data of this study shows higher frequency of non-cardia GC (52/120=43.3%) compared to cardia (39/120=32.3%). *H pylori* infection is a major risk factor of non-cardia GC [23]. It is responsible for almost 90% of non-cardia cancers [24].

In contrast to the previous results [16], this case control study shows that MTHFR C677T polymorphism increased the risk of GC by 35% (OR =1.35, 95% CI = 0.82 – 2.22), increase intestinal GC risk by 51% and diffused GC by 34% (table 2). This may be attributed to the sample size, geography, experiment design and ethnicity.

This study also showed that MTHFR C677T polymorphism interacted with *H pylori* infection is a significant risk factor among Jordanians. *H pylori* infection is responsible for 80% of distal gastric cancer cases, but not with cardia gastric carcinoma [25]. Positive association was also found between C677T polymorphism and an increased risk of intestinal-type GC [16]. A meta-analysis including of 134 studies (46,207 cases and 69,160 controls) showed an association between the MTHFR 677CT polymorphism and increased risk of esophageal and stomach cancer, especially among Asians [26]. This study also showed that MTHFR C677T and MTHFR 677 TT genotypes interacted with *H pylori* infection were significantly associated with increased risk of gastric cancer. Similar results were reported by Gao et al [27] who showed that the MTHFR TT genotype was significantly associated with 2.08 fold risk of gastric cancer when adjusting for potential risk factors. Subjects with TT and CT genotypes have lower enzymatic activity, 30% and 65%; respectively, compared to those with CC genotype, so less enzymatic activity lower S-adenosyl-Lmethionine and hypomethylation [28], which may be a clinical and reliable biomarker for early cancer detection.

In conclusion the results of this study and for the first time suggested *H pylori* infection modulate MTHFR C677T polymorphism and increased the risk for Jordanian GC, so *H pylori* eradication may be critical for primary prevention of GC.

## Acknowledgements

I would like to acknowledge Ibn-Nafees hospital staff for their support and helping in data collection and analysis. I would like also acknowledge Mrs. Asma Qudan for her helping in data collection and analysis.

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